Soil Biology and Biochemistry 120 (2018) 233–245

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Resilience of bacteria, archaea, fungi and N-cycling microbial guilds under plough and conservation tillage, to agricultural drought

A. Kaurin[a], R. Mihelič[a], D. Kastelec[a], H. Grčman[a], D. Bru[b], L. Philippot[b], M. Suhadolc[a,*]

[a] University of Ljubljana, Biotechnical Faculty, Center for Soil and Environmental Science, Jamnikarjeva 101, 1000 Ljubljana, Slovenia
[b] Agroécologie, INRA, AgroSup Dijon, Univ. Bourgogne Franche-Comté, F-21000 Dijon, France

ARTICLE INFO

Keywords:
Conservation agriculture
Ammonia-oxidising bacteria
Ammonia-oxidising archaea
Denitrifiers
Resilience
Climate change

ABSTRACT

Climate change causes droughts, which in turn cause significant physiological stress for soil microorganisms. In this study, we investigated how the abundance of total bacterial, crenarchaeal and fungal communities and the abundance of N-cycling microbial guilds responded to a severe agricultural drought event in a long-term experiment of minimum tillage (MT) and conventional ploughing (CT) at two soil depths. Drought, defined as a reduction of soil water content and increased soil temperature, significantly decreased the abundance of all the studied microbial communities. The data showed linear relationships between all dependent variables and soil water content and soil temperature for the examined range of soil water content (WHC 13–76%) and examined range of average daily soil temperature at 5 cm depth (17–30 °C). Thus, we found that the abundance of most studied microbial communities decreased by about 2% when water content decreased by 1 mass % and by about 10% when temperature increases by 1 °C. When comparing communities at average soil water content and average soil temperature, MT had higher average abundances of total bacterial and crenarchaeal 16S rRNA and fungal ITS genes in the 0–10 cm soil layer than did CT (1.9, 2.9 and 2.5 times, respectively), as well as AOA and AOB *amoA* (3.9 and 1.7 times, respectively), *nirK*, *nirS*, *nosZ*I and *nosZ*II genes (2.0, 1.8, 1.8 and 2.3 times, respectively); while significant differences between MT and CT in the 10–20 cm soil layer were found only in the average abundance of crenarchaeal 16S rRNA and crenarchaeal *amoA* genes (3.5 and 2.7 times greater under MT than CT). Regardless of the weather conditions during our study, the abundances of all communities were greater under MT 0–10 than under CT 0–10. After three weeks of severe drought, the greatest decrease in the abundance of all communities, bacterial and archaeal N-cycling guilds as well as total prokaryotes and fungi, was observed under MT 0–10. However, after only a few rainfall events, all communities under both tillage systems reached their initial abundance, demonstrating a high resilience.

1. Introduction

Climate change is expected to have a substantial impact on soil moisture and temperature conditions with enhanced frequency and duration of summer droughts (IPCC, 2013). Drought intensity (none, mild, moderate, severe, extreme) over vegetated lands (*agricultural drought*) is defined on the basis of soil water deficit causing water stress to the vegetation. In addition, drought frequency analysis is often used to estimate the return period or exceedance probability of a drought of given severity in comparison with the long-term normal (*reference*) state (Cammalleri et al., 2016). Models project decreases in soil water content from 5 to 15% in the upper 10 cm of the soil by the end of the century (Dai, 2012). Microbial communities and the biogeochemical cycles that they control have been shown to be affected by extreme soil water changes (Bapiri et al., 2010; Gleeson et al., 2010; Barnard et al., 2013; Placella and Firestone, 2013; Fuchslueger et al., 2014). It was thus shown that microbial composition was sensitive and not immediately resilient to a reduction in soil water content and/or increased temperature (Sheik et al., 2011; Gschwendtner et al., 2014; Keil et al., 2015). On the other hand, it has also been reported that soil microbial communities are resistant and resilient to climatic extremes, suggesting the presence of communities adapted to regular, seasonal fluctuations in temperature and rainfall experienced by such ecosystems (Griffiths et al., 2003; Cruz-Martínez et al., 2009; Barnard et al., 2013). Despite major scientific efforts, clear patterns of drought effects on biogeochemical cycles are rarely reported, possibly due to differences in (often) artificial experimental manipulations, different soil characteristics, as well as differences between studies in the drought intensities and previous drying-rewetting cycles (Banerjee et al., 2016; Canarini et al., 2017).

* Corresponding author.
E-mail address: marjetka.suhadolc@bf.uni-lj.si (M. Suhadolc).

https://doi.org/10.1016/j.soilbio.2018.02.007
Received 30 June 2017; Received in revised form 7 January 2018; Accepted 9 February 2018
0038-0717/ © 2018 Elsevier Ltd. All rights reserved.

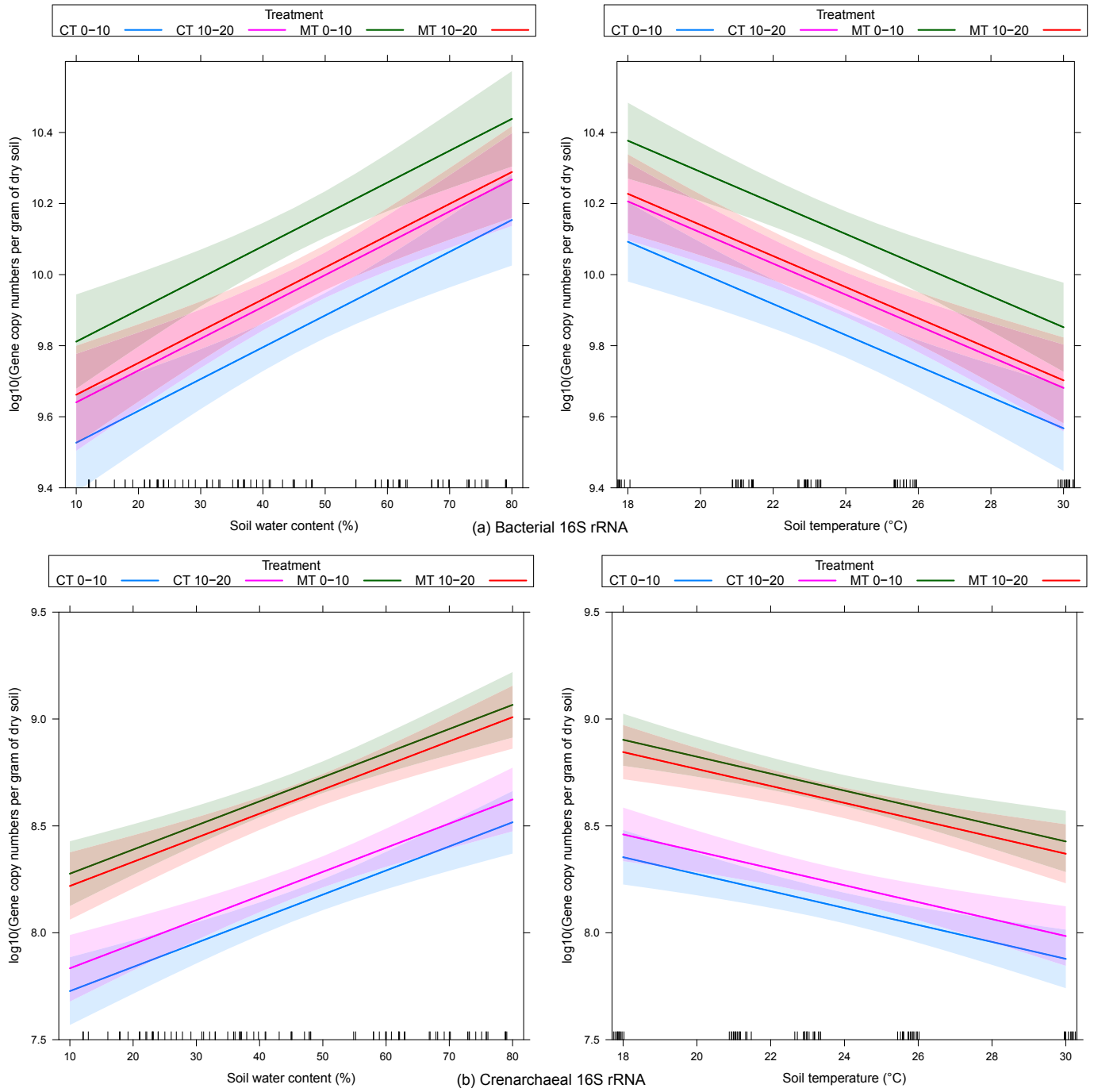


Fig. 1. Effect plots for (a) bacterial 16S rRNA, (b) crenarchaeal 16S rRNA, and (c) fungal ITS; nitrifying (d) bacterial and (e) archaeal *amoA*; and denitrifying (f) *nirK*, (g) *nirS*, (h) *nosZ*I and (i) *nosZ*II gene copies g^{-1} soil in dependence of soil water content (expressed as % of WHC) at average soil temperature (left) and in dependence on soil temperature (°C) at average soil water content (right) at 0–10 cm and 10–20 cm soil layers under conventional tillage (CT) and minimum tillage (MT).

Many researchers have proposed the replacement of traditional tillage (ploughing) with conservation tillage to improve soil water storage and increase water use efficiency (reviewed by Busari et al., 2015). Conservation tillage, with reduced soil disturbances and improved residue management, has been shown to result in an accumulation of soil organic carbon (SOC) in the very topsoil, leading to improved soil structure, aggregate stability, water infiltration and water holding capacity (Yaduvanshi and Sharma, 2008; Tabaglio et al., 2009; Perez-Brandán et al., 2012). Moreover, the abundance and microbial community composition have also been reported to respond to the stratification of SOC and nutrients under conservation tillage (Kaisermann et al., 2013; Kaurin et al., 2015). However, to the best of our knowledge, the impact of severe drought on soil microbial communities, especially those responsible for key soil functions, under different tillage regimes is still largely unknown.

The present study focused on the key players of the nitrogen cycle, ammonia oxidizers (nitrifiers) and denitrifiers, since they have both recently been proposed to be model functional guilds for assessing microbial response to climate change (Thion and Prosser, 2014; Gschwendtner et al., 2014, respectively). Ammonia oxidizers drive the first, rate limiting step of nitrification (Prosser and Nicol, 2008), while denitrifiers are phylogenetically more heterogeneous and are capable of reducing soluble nitrogen oxides into the gasses N_2O and N_2 (Philippot et al., 2007).

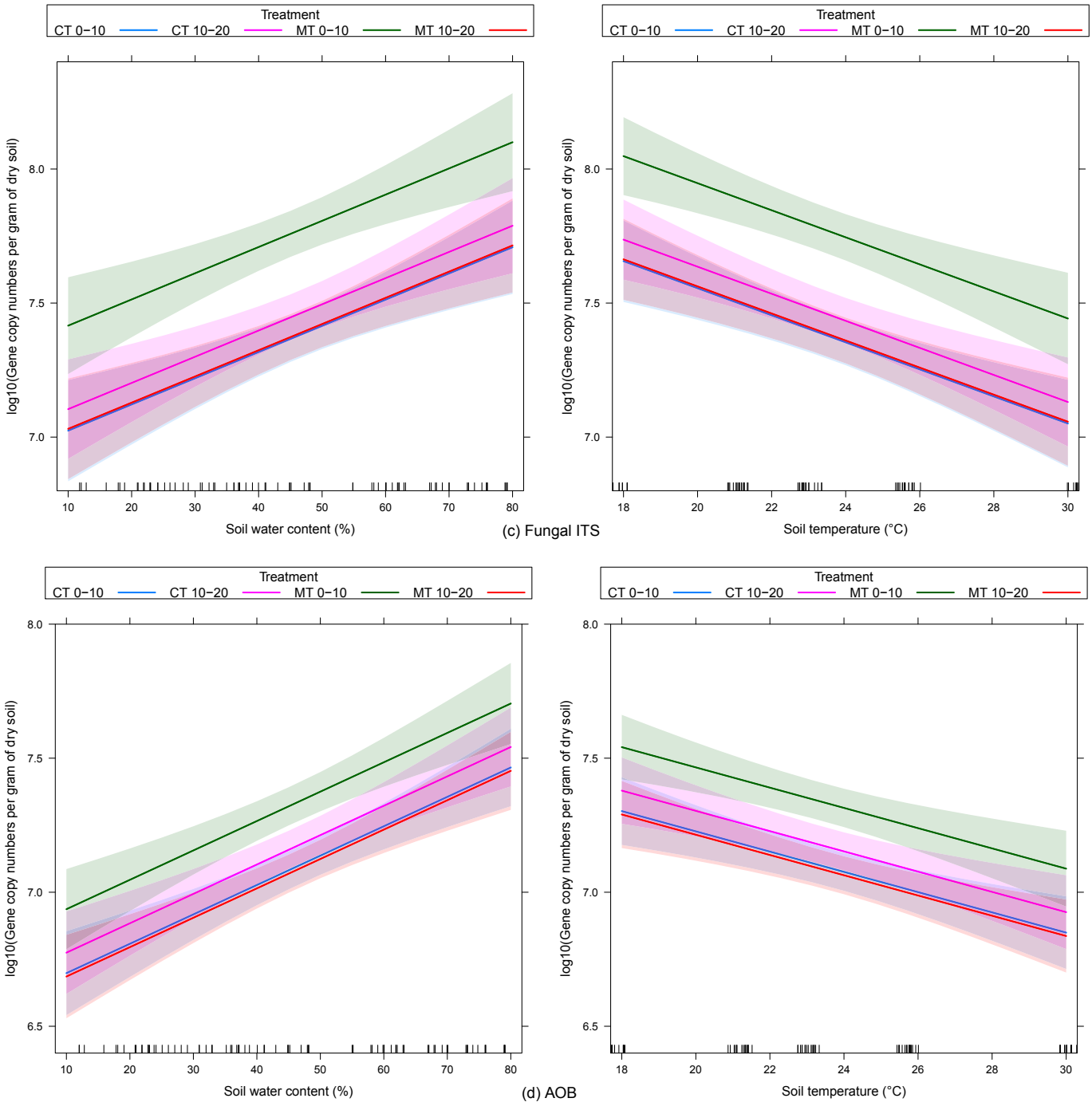


Fig. 1. (*continued*)

The aims of this study were to assess the impact of severe agricultural drought event on nitrifying and denitrifying communities by quantifying *amoA*, *nirK*, *nirS, nosZ*I and *nosZ*II genes in a long-term experiment of conventional (CT) and minimum tillage (MT) at two depths. The drought event was indicated as severe, while the cumulative index of drought stress was the highest during the last fifty years in Slovenia (Sušnik and Valher, 2014). Under examined field conditions the soil water contents decreased up to 13% of water holding capacity (WHC) and the average daily soil temperatures at 5 cm depth increased up to 30 °C. We hypothesised (i) that the effects of drought might be greater on ammonia oxidizers than on denitrifiers as denitrification is a phylogenetically broader process and (ii) that the effects of drought might be less pronounced under MT than under CT due to the higher soil organic matter contents in the top soil.

2. Materials and methods

2.1. Field site and experimental design

The study site is located in Moškanjci near Ptuj in the sub-Pannonian basin, Slovenia. The regional climate is continental, with an average annual temperature of 10.6 °C and average precipitation of 913 mm year^{-1} for the 1999–2013 period. Soils developed on the Drava river alluvial plain and classified as Skeletic, Eutric Cambisol, are shallow (40–60 cm), with a high gravel content (up to 15% in the upper 0–20 cm layer) and loamy texture (30–40% sand, 42–48% silt and 17–26% clay), and pH 6.2.

The field experiment was established in 1999 and consisted of two tillage systems, conventional mouldboard ploughing (CT) and

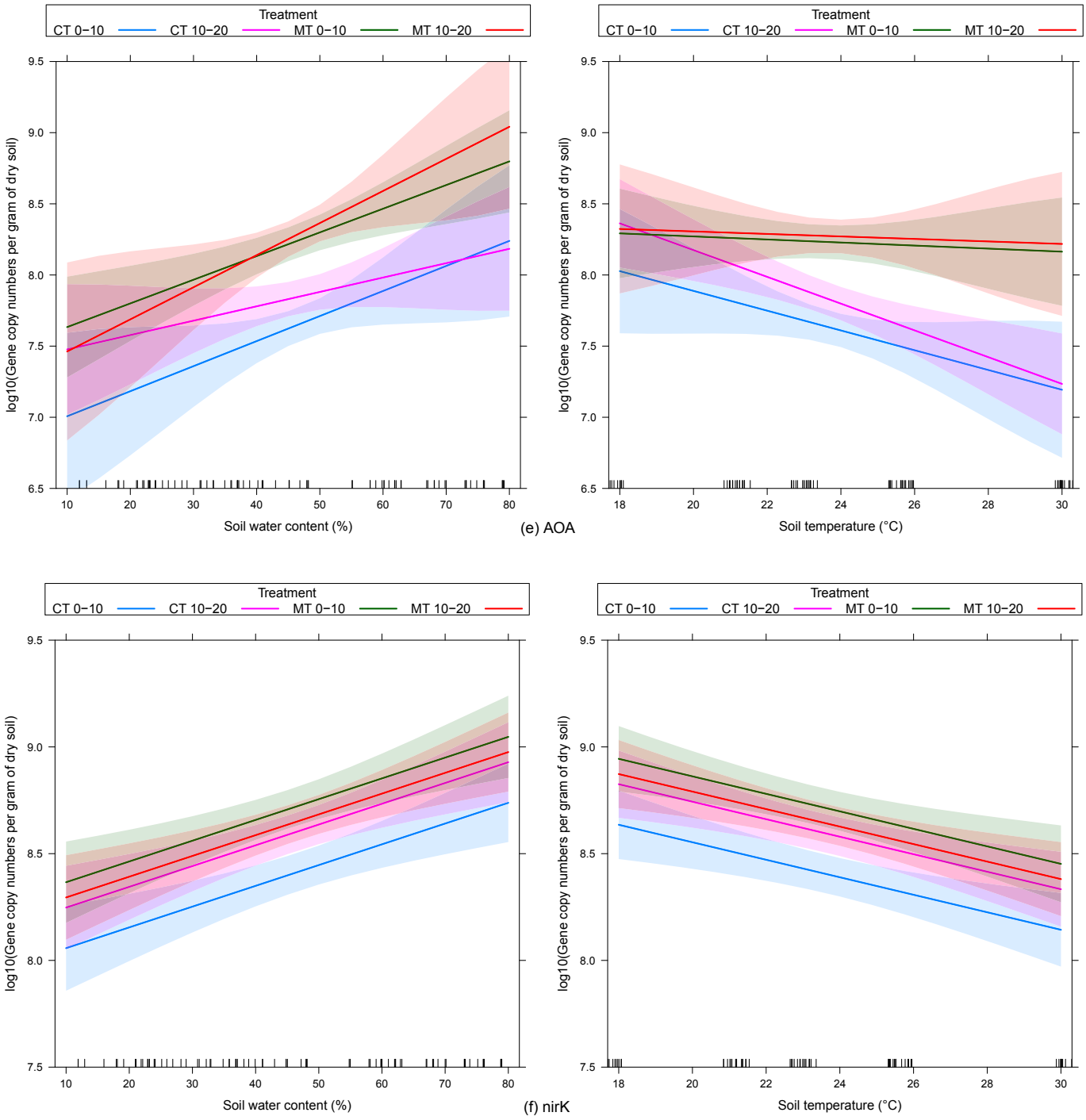
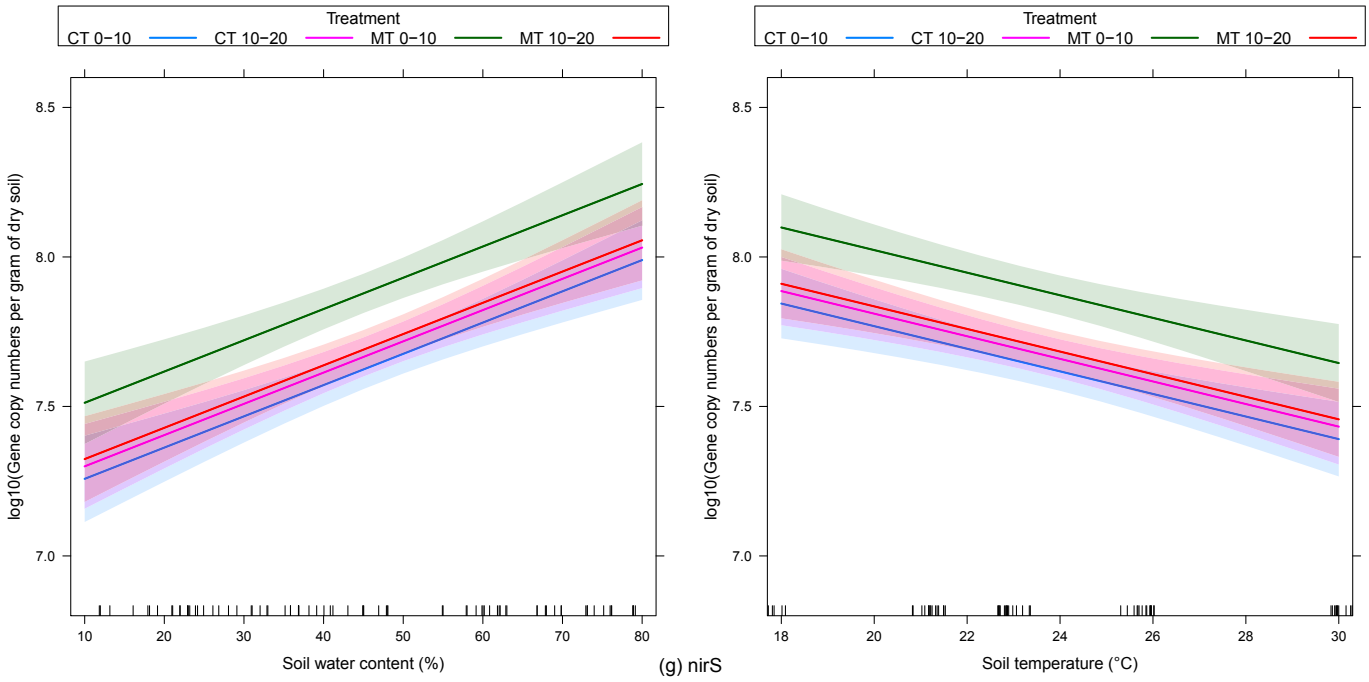


Fig. 1. (*continued*)

minimum tillage (MT). Each management treatment consisted of four replicated plots. The size of each plot was 12 × 8 m and there was a 1 m buffer zone between the tillage treatments. In the CT treatment, ploughing was done with a mouldboard plough 20 cm deep, while in the MT treatment, soil disturbance was done up to 10 cm deep with a special disc harrow (Evers Agro, Senner), leaving at least 30% of plant residues on the soil surface at the time of planting. For a detailed description of the experimental design and soil properties see Kaurin et al. (2015).

2.2. Weather conditions

Precipitation, air temperature, soil temperature at 5 cm depth and soil water contents at 10, 20 and 40 cm were recorded continuously by an on-site iMETOS electronic weather station. The soil sampling scheme was based on soil water conditions and was as follows: 10th July (before drought), 25th July (dry, 2 weeks without rain), 7th August (dry, 4 weeks without rain), 12th August (rewetted, after the first rain) and 3rd September (rewetted, after 3 weeks of usual rainfall events) (Fig. S1). It is important to note that soil water contents were determined in all samples individually; exact data for each plot and sampling time combination were thus used for further evaluations. Soil temperature was measured only by the weather station (1 sensor at 5 cm soil depth per experiment) on an hourly basis, and average daily temperature was used in the statistical models.



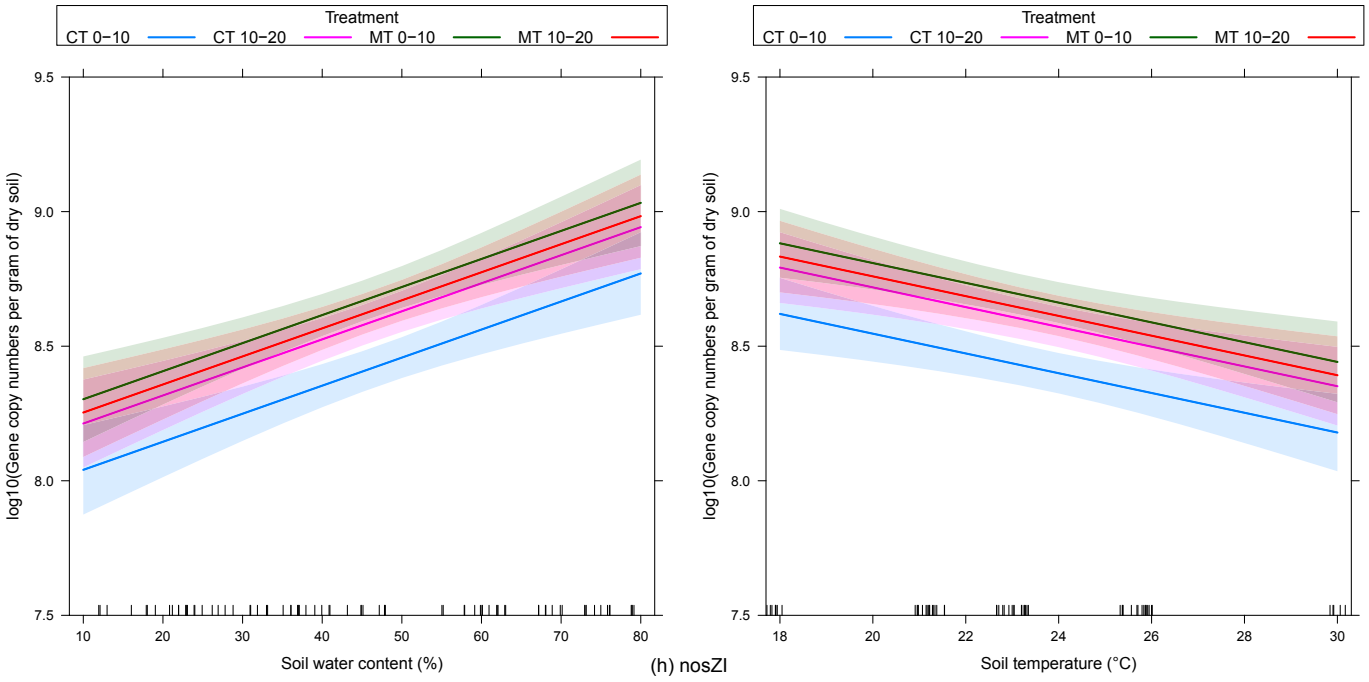


Fig. 1. (*continued*)

2.3. Soil sampling and soil water content determination

Soil samples were taken 5 times during summer 2013 (Fig. S1) from four replicated plots per treatment. A composite soil sample was taken per plot (5 soil cores of 3 cm diameter) at two soil layers: 0–10 cm (tilled horizon in MT, ploughed horizon in CT) and 10–20 cm (non-tilled horizon in MT, ploughed horizon in CT). Samples were homogenized and sieved through a 2 mm pore-size mesh sieve to remove roots and stones. One part of the homogenized soil sample was placed on dry ice and stored at −20 °C for molecular analysis and the other part was dried at 40 °C for 24 h and used for chemical analysis.

Soil water content (WC) was determined in each homogenized soil sample by drying at 105 °C to constant weight (ISO 11465, 1993) and expressed as % of water holding capacity (WHC). WHC was measured on a pressure plate by water extraction at −33 kPa (ISO 11274, 1998).

2.4. Soil chemical analysis

Dissolved organic carbon (DOC), dissolved organic nitrogen (DON), nitrate nitrogen (NO_3-N) and ammonium nitrogen (NH_4-N) were extracted with 0.01M $CaCl_2$ at a soil: liquid ratio of 1:10, and determined using a vario TOC cube (Elementar, Germany) for DOC and DON, and a Gallery Automated Photometric Analyzer (Thermo Scientific) for NO_3-N and NH_4-N detection.

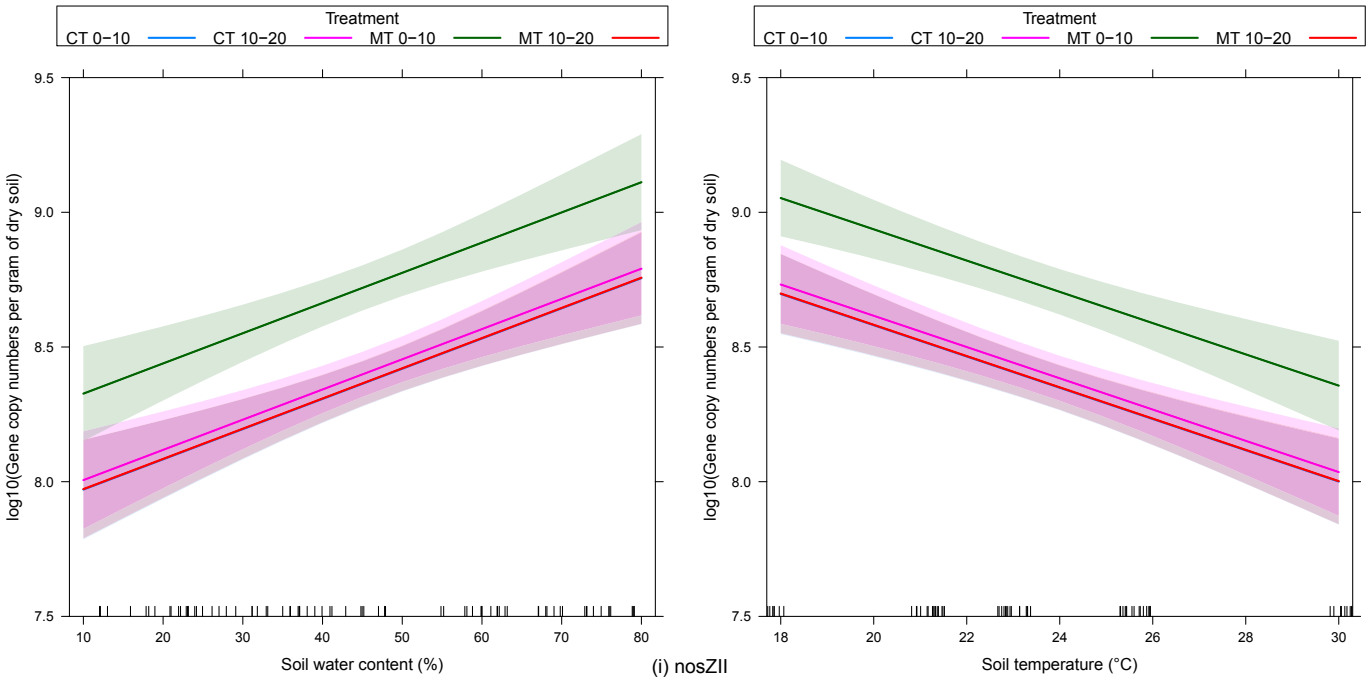


Fig. 1. (*continued*)

2.5. Quantitative real-time PCR (qPCR)

For nucleic acid extraction, a PowerSoil RNA Isolation Kit was used in combination with an RNA PowerSoil DNA elution accessory kit, following the manufacturer's instructions (MoBIO Laboratories, USA). The extracted RNA was not of sufficient quality for reliable gene expression results, so further analysis proceeded only with total extracted DNA. DNA concentrations were quantified using a Quant-iT DNA Assay Kit (Life Technologies), diluted to 1 ng μl^{-1} and stored at −20 °C until use. The presence of PCR inhibitors in DNA samples was established using an exogenous external control (the pGEM-T vector, Promega) by diluting and spiking soil DNA with a known amount of pGEM-T vector before qPCR. Inhibition was not detected. To ensure PCR specificity, melting curves were analysed for all runs.

The abundances of genes encoding catalytic enzymes of ammonia-oxidizers and denitrifiers were assessed by using qPCR by targeting bacterial and crenarchaeal *amoA* (Leininger et al., 2006; Tourna et al., 2008), *nirK* (Henry et al., 2004), *nirS* (Kandeler et al., 2006), *nosZ*I and *nosZ*II genes (Henry et al., 2006; Jones et al., 2013). The abundance of total bacterial and crenarchaeal 16S rRNA (Muyzer et al., 1993; Ochsenreiter et al., 2003), and of fungal ITS genes (White et al., 1990) were also determined. Reactions were carried out in a 15 µl reaction volume containing 1 µM of each primer, 250 ng of T4gp32 (MP Biomedicals), 7.5 µl ABsolute QPCR SYBR Green Rox mix (Thermo Scientific) and 2 µl of DNA template using ABI Viia7 Software (Applied Biosystems, USA). All primers and PCR conditions are described in the supplementary material (Table S1).

Absolute quantification was carried out based on standard curves; 10-fold serial dilutions (10^7 to 10^2 gene copies per reaction) of plasmid standards containing cloned segment of target genes were assessed.

The results were expressed in absolute gene abundance, calculated as copy numbers per gram of dry soil. Additionally, we calculated the proportion of ammonia-oxidizers and denitrifiers to total bacterial or crenarchaeal communities to estimate the relative abundance of functional N-cycling genes in the total community.

2.6. Statistical analysis

Data for the abundance of 16S rRNA, ITS, nitrification and denitrification genes were collected in a completely randomized field experiment considering two different tillage treatments (CT, MT), two soil layers (0–10 cm, 10–20 cm) and two environmental covariables (soil moisture and soil temperature). Values for soil water content, expressed as a percentage of soil water holding capacity, and the average soil temperature measured at 5 cm depth, were mean-centred. Linear mixed models were used to analyse the dependence of variables illustrating soil microbial structure on two fixed factors (tillage, soil depth) and their interaction. The two numerical covariables, soil water content and soil temperature, together with the interactions of each one with tillage and soil depth, were also included in the model. The measurements were done on the same experimental unit (plot) at different consecutive times during the vegetation period, the plot was therefore included in the linear model as a random effect. All dependent variables were $\log_{10}$ transformed due to heteroscedasticity. The log transformed data showed a linear relationship between all dependent variables and soil water content, as well as soil temperature, for the given range of soil water content (76%–13% of WHC) and for the given range of average daily soil temperature (17 °C-30 °C). For better interpretability, $\log_{10}$ transformation of the dependent variables was used for graphical presentation (Fig. 1) and ln transformation for the linear model parameter estimation (Tables 1 and 2). The interaction between tillage and soil depth was statistically significant in all cases. Further analysis was therefore done considering one fixed factor “treatment”, with four levels (CT 0–10, CT 10-20, MT 0-10, MT 10–20), determined with all combinations between two levels of tillage and two levels of soil depth, instead of tillage, soil depth and their interaction. For all the dependent variables, except AOA, the interactions between treatment and soil water content and between treatment and soil temperature were statistically non-significant and were therefore excluded from the final linear mixed model. A model matrix was constructed for each response variable, six null hypotheses were thus tested on the basis of estimated model parameters and their variance-covariance matrix simultaneously. The first four parameters in the model estimate the differences between averages of the variables of four treatments (CT 10–20 *vs.* CT 0-10, MT 10–20 vs. MT 0-10, MT 0–10 *vs.* CT 0–10, and MT 10–20 *vs.* CT 10–20 in Table 1) at average water holding capacity and at average soil temperature. The fifth parameter in the model estimates the effect of soil water on considered responses for all four

Table 1
p-values from ANOVA table for linear mixed models (upper part) and results of the simultaneous null hypothesis testing (lower part). Four treatments were defined with tillage (conventional tillage (CT) and minimum tillage (MT)) and soil depth (0–10 and 10–20 cm). All variables were log transformed prior the analysis.

Source of variation	Total extracted DNA		Bacterial 16S rRNA		Crenarchaeal 16S rRNA		ITS		AOB *amoA*		AOA *amoA*		*nirK*		*nirS*		*nosZI*		*nosZII*	
Treatment	< 0.001		0.006		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001	
Water content (WC)	< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001	
Temperature	< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001	
Treatment:WC	0.114		0.832		0.121		0.755		0.263		0.032		0.531		0.091		0.468		0.276	
Treatment:Temperature	0.372		0.414		0.228		0.720		0.227		0.108		0.637		0.690		0.638		0.189	
Contrasts:																				
CT 10–20 - CT 0-10	0.693	+	0.061		0.173		0.608		0.4774		0.146	+	0.020		0.868	+	0.009		0.971	
MT 10–20 - MT 0-10	< 0.001	–	0.006		0.739	–	< 0.001	–	< 0.001		0.999		0.749	–	< 0.001		0.857	–	< 0.001	
MT 0–10 - CT 0-10	< 0.001	+	< 0.001	+	< 0.001	+	< 0.001	+	< 0.001	+	< 0.001	+	< 0.001	+	< 0.001	+	< 0.001	+	< 0.001	
MT 10–20 - CT 10-20	0.253		0.988	+	< 0.001		0.685		0.320	+	< 0.001		0.933		0.983		0.925		0.977	
WC slope	< 0.001	+	< 0.001	+	< 0.001	+	< 0.001	+	< 0.001	+	[a]	+	< 0.001	+	< 0.001	+	< 0.001	+	< 0.001	
T slope	< 0.001	–	< 0.001	–	< 0.001	–	< 0.001	–	< 0.001	–	[b]	–	0.004	–	< 0.001	–	0.002	–	< 0.001	

+ or – represents the sign of estimated statistically significant effects: (i) for the differences between averages for two treatments, i.e. the average Total extracted DNA at MT 10–20 is significantly lower than the average Total extracted DNA at MT 0–10, while the estimated difference of averages is negative; (ii) if the slope is positive this means that the average abundance increases if WC/Temperature increases, while the interactions between Treatment and WC and Treatment and Temperature are not statistical significant, the effect of WC/Temperature is for all 4 treatments the same.

[a] The effect of WC is statisticaly significant ($p = 0.010$) only for MT 0-10.

[b] The effect of T is statisticaly significant ($p = 0.006$) only for CT 10-20.

treatments, considering constant soil temperature (WC slope in Tables 1 and 2). The last parameter in the model estimates the effect of soil temperature on considered responses for all four treatments at constant water holding capacity (T slope in Tables 1 and 2). Only for the AOA, the interaction terms were statistically significant and it was therefore left in the final model. In this case twelve null hypothesis were tested simultaneously, the slope for the effect of WC for each treatment and the slope for the effect of T for each treatment additionally. The "lmer" function from the package "lme4" was used for linear mixed modelling and the "glht" function from the package "multcomp" for simultaneous testing of six in all except one model. All statistical analyses were performed using R program version 3.3.2 (R Core Team, 2017).

3. Results and discussion

The studied long term experimental site, established in 1999 to compare minimum tillage and conventional mouldboard ploughing, experienced one of the naturally occurring extreme droughts from late July to mid August 2013. It is important to note that the drought conditions were very close to the most severe drought recorded during the last fifty years in Slovenia (Sušnik and Valher, 2014). The cumulative meteorological water balance for the period from April 1st to August 31st in the examined Dravsko-Ptujsko polje region showed that the water balance in comparison to the long-term average (1961–2012) exceeded extreme drought conditions in the summer period. Percentile analysis thus showed that after July 20th, the water deficit reached the 75th percentile (severe drought conditions) and, in some days at the beginning of August, almost the 90th percentile (extreme drought conditions). The cumulative water balance was - 304 mm instead of the average - 83 mm, while the cumulative index of drought stress (DISSk) was almost three times higher in 2013 than the average for the period 1961–2012 (Sušnik, 2014; ARSO, 2017). The no-precipitation period, combined with increasing soil temperatures, resulted in reduced soil water content. Soil was supplied with plant available water until July 18th, drought then started to occur, with a soil moisture tension of ca. 50 kPa (Fig. S1). The soil moisture deficit increasingly intensified when the soil water tension rose above 100 kPa, on July 22nd at a 10 cm depth, a few days later also at a 20 cm depth, and after July 25th until August 8th above the maximum measurable water tension under field conditions (200 kPa). Under MT, the soil water content dropped from 76% to 13% of WHC in the 0–10 cm and from 62% to 24% in the 10–20 cm layer; and under CT, from 67% to 20% and from 69% to 24%, respectively. Soil dryness coincided with high soil temperatures, the average daily temperature at a 5 cm soil depth increased from 22 °C to 30 °C with daily maximum above 35 °C (max 37.7 °C) from July 28th until August 8th. After only a few rainfall events during a three-week period, the soil water content and soil temperature returned to their initial values, allowing an assessment of the resilience of microbial communities to this drought event.

3.1. Impact of tillage and soil depth on microbial communities

The abundance of total bacterial, crenarchaeal and fungal communities, and the abundance of N-cycling microbial guilds were significantly affected by tillage and depth, as well as by the soil water content and temperature (Table 1). In the upper 0–10 cm soil layer, the abundances of all studied communities, at average soil water content and temperature, were significantly higher under MT than CT. The same effect of the tillage regime was observed in the lower 10–20 cm soil layer but to a lesser extent with significant differences found only for total crenarchaeal 16S rRNA and AOA. One of the major reasons for higher microbial abundance under MT 0–10 than CT 0–10 is likely to be the higher organic carbon content, total organic carbon (1.60 ± 0.07% and 1.45 ± 0.05%, respectively) and dissolved organic carbon (DOC) (Table S2, S3). Several other soil properties, such as soil structure and water retention characteristics, were also improved in the topsoil of MT

Table 2
Linear model estimates of average values of analysed variables at average soil water content (WC) and average soil temperature (T) with 95% confidence intervals, for four treatments defined with tillage and soil depth (conventional tillage (CT) and minimum tillage (MT)) and soil depth (0–10 and 10–20 cm); the estimates of the effect of WC and T (WC slope and T slope) with 95% confidence intervals.

Total extracted DNA and abundance of general bacterial, archaeal and fungal communities

Treatment	Total extracted DNA g^{-1} soil			Bacterial 16S rRNA copies g^{-1} soil			Crenarcheal 16S rRNA copies g^{-1} soil			Fungal ITS copies g^{-1} soil		
	Average	LCL 95%	UCL 95%	Average	LCL 95%	UCL 95%	Average	LCL 95%	UCL 95%	Average	LCL 95%	UCL 95%
CT 0–10 cm	17.93	15.18	21.18	$7.07\ 10^9$	$5.63\ 10^9$	$8.88\ 10^9$	$1.44\ 10^8$	$1.08\ 10^8$	$1.93\ 10^8$	$2.38\ 10^7$	$2.86\ 10^7$	$3.04\ 10^7$
CT 10–20 cm	20.09	17.01	23.72	$9.18\ 10^9$	$7.12\ 10^9$	$11.53\ 10^9$	$1.67\ 10^8$	$1.24\ 10^8$	$2.25\ 10^8$	$2.86\ 10^7$	$2.24\ 10^7$	$3.66\ 10^7$
MT 0–10 cm	34.94	29.57	41.28	$13.60\ 10^9$	$10.83\ 10^9$	$17.08\ 10^9$	$4.21\ 10^8$	$3.03\ 10^8$	$5.87\ 10^8$	$5.86\ 10^7$	$4.58\ 10^7$	$7.49\ 10^7$
MT 10–20 cm	24.04	20.36	28.39	$9.65\ 10^9$	$7.69\ 10^9$	$12.12\ 10^9$	$5.86\ 10^8$	$4.20\ 10^8$	$8.16\ 10^8$	$2.41\ 10^7$	$1.89\ 10^7$	$3.09\ 10^7$
WC slope	0.018	0.009	0.028	0.021	0.010	0.031	0.026	0.014	0.038	0.023	0.009	0.036
T slope	−0.102	−0.148	−0.056	−0.101	−0.150	−0.052	−0.091	−0.147	−0.035	−0.116	−0.183	−0.049

Abundance of Ammonia-oxidizers

	AOB *amoA* copies g^{-1} soil			**AOA** *amoA* copies g^{-1} soil		
	Average	LCL 95%	UCL 95%	Average	LCL 95%	UCL 95%
CT 0–10 cm	$1.24\ 10^7$	$1.01\ 10^7$	$1.52\ 10^7$	$4.39\ 10^7$	$3.09\ 10^7$	$6.25\ 10^7$
CT 10–20 cm	$1.48\ 10^7$	$1.20\ 10^7$	$1.81\ 10^7$	$6.93\ 10^7$	$4.87\ 10^7$	$9.85\ 10^7$
MT 0–10 cm	$2.15\ 10^7$	$1.75\ 10^7$	$2.64\ 10^7$	$17.05\ 10^7$	$11.98\ 10^7$	$24.27\ 10^7$
MT 10–20 cm	$1.20\ 10^7$	$0.98\ 10^7$	$1.48\ 10^7$	$18.97\ 10^7$	$13.33\ 10^7$	$26.98\ 10^7$
WC slope	0.025	0.014	0.037	0.038[a]	0.006[a]	0.070[a]
T slope	−0.087	−0.142	−0.032	−0.216[b]	−0.389[b]	−0.044[b]

Abundance of Denitrifiers

	nirK copies g^{-1} soil			***nirS*** copies g^{-1} soil			***nosZI*** copies g^{-1} soil			***nosZII*** copies g^{-1} soil		
	Average	LCL 95%	UCL 95%	Average	LCL 95%	UCL 95%	Average	LCL 95%	UCL 95%	Average	LCL 95%	UCL 95%
CT 0–10 cm	$2.54\ 10^8$	$1.63\ 10^8$	$3.96\ 10^8$	$4.28\ 10^7$	$3.54\ 10^7$	$5.17\ 10^7$	$2.61\ 10^8$	$1.91\ 10^8$	$3.56\ 10^8$	$2.37\ 10^8$	$1.86\ 10^8$	$3.01\ 10^8$
CT 10–20 cm	$3.96\ 10^8$	$5.54\ 10^8$	$6.17\ 10^8$	$4.74\ 10^7$	$3.93\ 10^7$	$5.73\ 10^7$	$3.87\ 10^8$	$2.84\ 10^8$	$5.28\ 10^8$	$2.57\ 10^8$	$2.02\ 10^8$	$3.27\ 10^8$
MT 0–10 cm	$5.17\ 10^8$	$3.32\ 10^8$	$8.05\ 10^8$	$7.71\ 10^7$	$6.38\ 10^7$	$9.32\ 10^7$	$4.76\ 10^8$	$3.49\ 10^8$	$6.49\ 10^8$	$5.38\ 10^8$	$4.23\ 10^8$	$6.84\ 10^8$
MT 10–20 cm	$4.42\ 10^8$	$2.81\ 10^8$	$6.88\ 10^8$	$5.01\ 10^7$	$4.15\ 10^7$	$6.05\ 10^7$	$4.26\ 10^8$	$3.12\ 10^8$	$5.81\ 10^8$	$2.38\ 10^8$	$1.87\ 10^8$	$3.02\ 10^8$
WC slope	0.022	0.008	0.037	0.024	0.014	0.035	0.022	0.008	0.037	0.026	0.012	0.039
T slope	−0.094	−0.165	−0.024	−0.087	−0.138	−0.0360	−0.094	−0.165	−0.024	−0.134	−0.199	−0.068

[a] The effect of WC is statisticaly significant (p = 0.010) only for MT 0–10.
[b] The effect of T is statisticaly significant (p = 0.006) only for CT 10-20.

in comparison to CT (Kaurin et al., 2015), which is in agreement with the literature (Helgason et al., 2009; Sun et al., 2016; White and Rice, 2009). Recent results of Krauss et al. (2016) show increases (2.9 and 2.5 times, respectively) of AOA and AOB *amoA* gene abundances in the topsoil of MT compared with CT. We also observed statistically significant higher abundance of both AOA and AOB in the topsoil of MT in comparison to CT (Tables 1 and 2), together with an increased NO_3^- content, which suggests higher nitrification in this layer (Table S3). Our data showed that conservation tillage (MT) also led to statistically significant increase in the denitrifying community, with about 2 fold higher *nirK*, *nirS*, *nosZ*I, and *nosZ*II gene abundances, at average soil WC and T, under MT 0–10 than under CT 0–10 (Tables 1 and 2). Higher denitrifier abundances under conservation tillage have already previously been reported (Baudoin et al., 2009; Attard et al., 2011).

In parallel with the significant increase of N-cycling microbial guilds, as well as prokaryotes and fungal abundance under MT in comparison to CT, shifts in their composition due to the tillage regime were also observed, although less prominent. When comparing stratified soil layers of MT, significantly higher bacteria to fungi and crenarchaea to fungi ratios were found in the lower layer than in the topsoil (Table 2). While a decrease of fungal biomass with tillage intensity has been reported in several studies, due to disruption of fungi mycelia by tillage (Wardle, 1995; Helgason et al., 2010), we did not observe any significant difference between CT and MT in the 10–20 layer. Helgason et al. (2009) found an effect of tillage on fungal biomass in the deeper layer in only half of studied sites. More recently, Sun et al. (2016) even reported a higher fungal biomass under mouldboard ploughing compared to no-till in the deeper horizon. Such a proliferation of fungi in the topsoil of reduced tillage in comparison to the lower layer could be explained by access to more resources closer to the surface in the reduced tillage treatment due to stratification of organic matter inputs, which may outweigh the cost of physiological damage inflicted by small scale mechanical disturbance as recently reported by Hydbom et al. (2017).

Consistent with previous research on ammonia-oxidizers (Leininger et al., 2006; Adair and Schwartz, 2008; Bru et al., 2011; He et al., 2007; Sims et al., 2012; Strauss et al., 2014), AOA dominated over AOB under all treatments (Table 2). Our results showed that the AOA to AOB ratio was significantly higher under MT than CT, especially in the lower soil layer, because of decreasing AOB and unchanged AOA abundance with soil depth (Tables 1 and 2), which is in agreement with Leininger et al. (2006). However, the relative contribution of bacteria and archaea to the first step of nitrification is still unclear and further work is still needed to determine what is carrying out nitrification under a given combination of soil conditions (He et al., 2007; Prosser and Nicol, 2008, 2012; Jia and Conrad, 2009; Gubry-Rangin et al., 2010).

We also found that the *nosZI*/*nosZ*II ratio was significantly affected by soil depth but not the tillage regime. Changes in the *nosZI*/*nosZ*II ratio were due to a significantly lower abundance of *nosZ*II in the lower soil layer (Table 2). Recent studies have suggested niche separation between the two N_2O reducing communities (Jones et al., 2013; Domeignoz-Horta et al., 2015); with the *nosZ*II community being more sensitive to environmental changes than *nosZ*I. Agriculture is the main source of terrestrial N_2O emissions, a potent greenhouse gas and the main cause of ozone layer depletion. It has been suggested that the *nosZ*II community rather than *nosZ*I is of importance for the soil N_2O

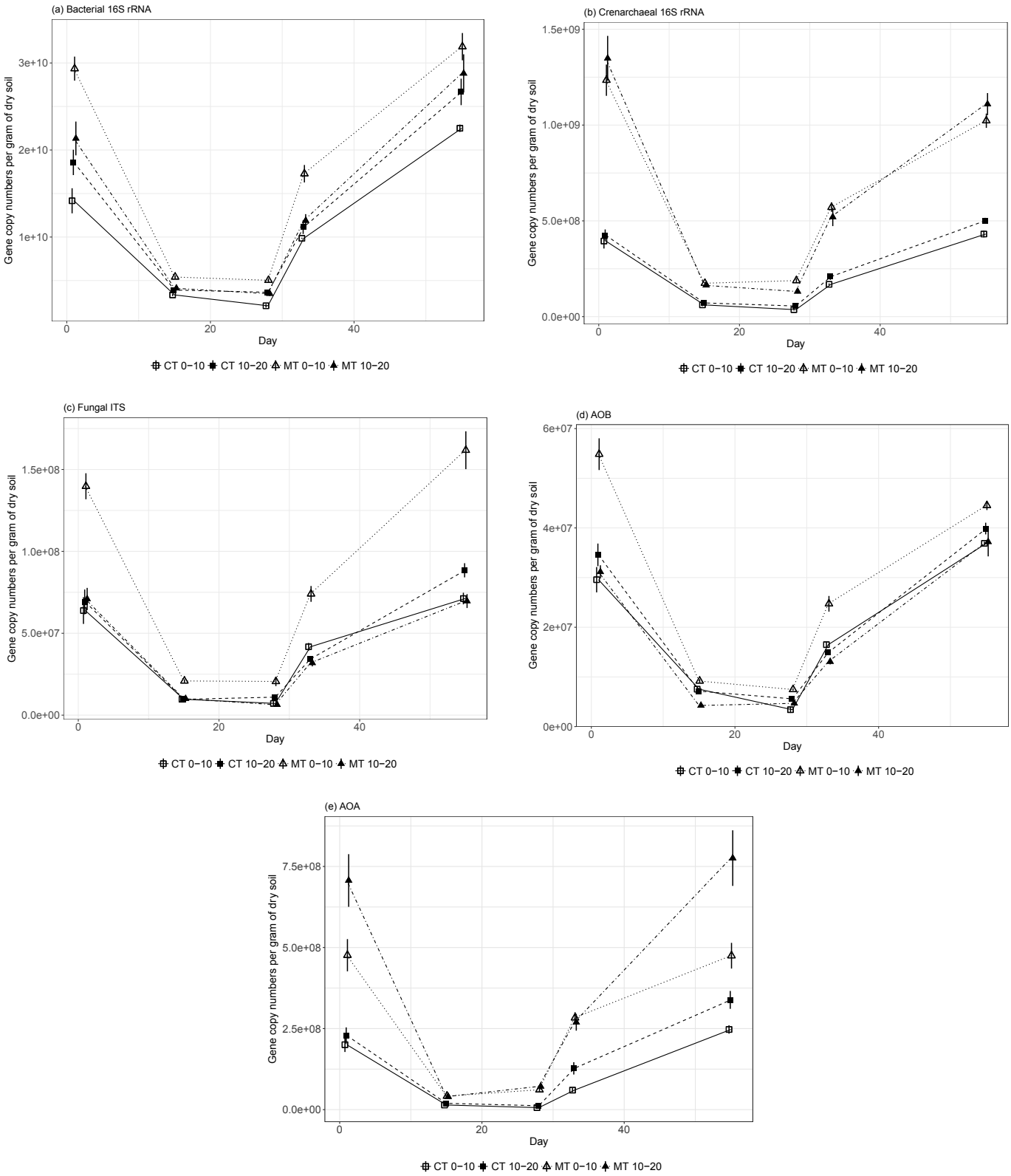


Fig. 2. Average (a) bacterial 16S rRNA, (b) crenarchaeal 16S rRNA, and (c) fungal ITS; nitrifying (d) bacterial and (e) archaeal *amoA*; and denitrifying (f) *nirK*, (g) *nirS*, (h) *nosZ*I and (i) *nosZ*II gene copies at 0–10 cm and 10–20 cm soil layers under conventional tillage (CT) and minimum tillage (MT) during the summer 2013: day 1 (10th July - normal conditions), day 15 (25th July - two weeks without rain), day 28 (7th August - four weeks without rain), day 33 (12th August - after the first rain) and day 55 (3rd September - after three weeks of usual rainfall events). Averages with standard errors are shown.

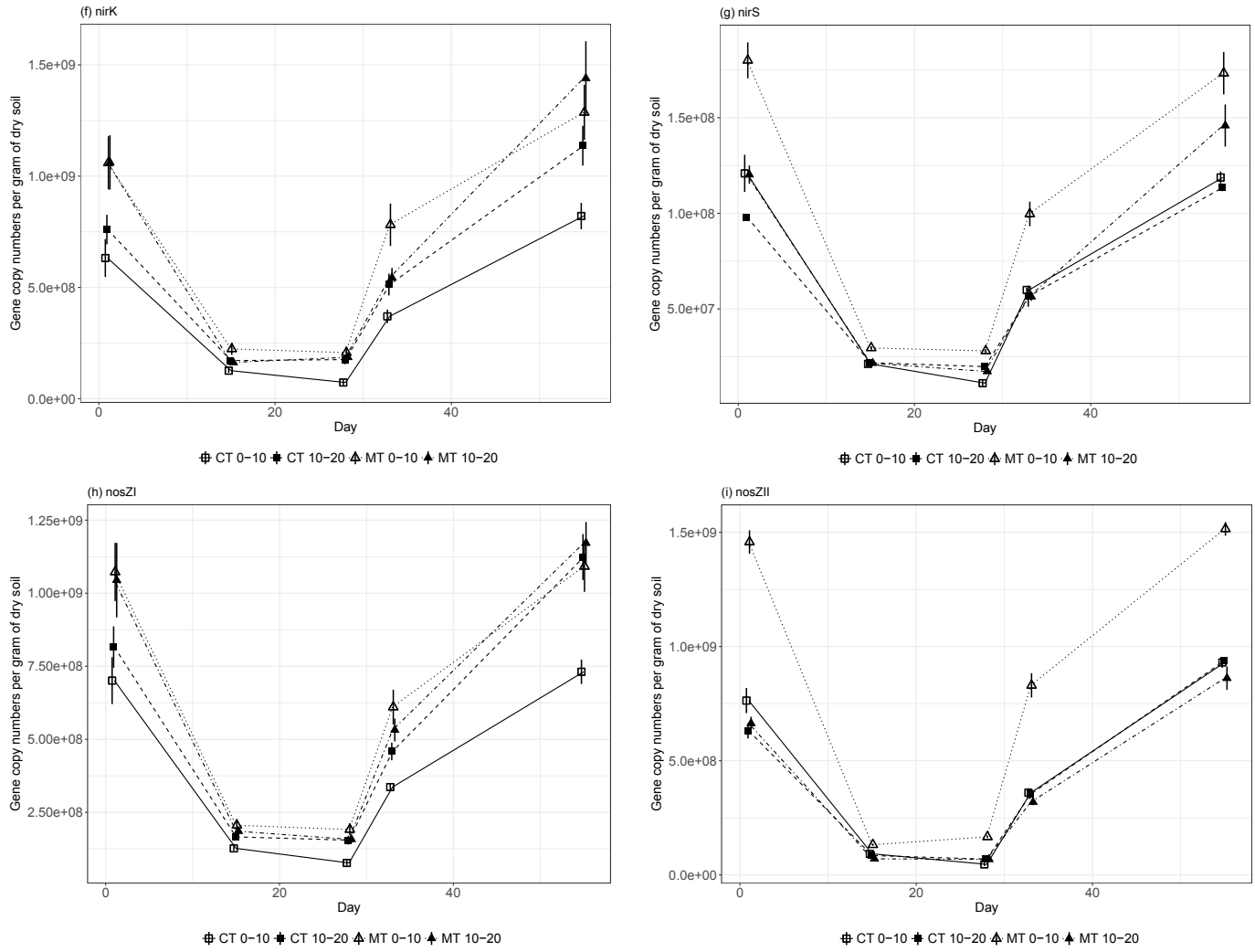


Fig. 2. (*continued*)

sink capacity (Jones et al., 2013; Domeignoz-Horta et al., 2016). The observed shift in the *nosZI*/*nosZ*II ratio might therefore have consequences for N_2O emissions between soil layers, with a stronger N_2O sink capacity in the upper layer.

3.2. Impact of drought on microbial communities

Drought, defined as a reduction of soil water content and increased soil temperature, significantly decreased the abundance of all studied microbial communities (Tables 1 and 2). The extent of the decrease was very similar among communities. The negative linear effect of soil temperature, at constant soil water content, and the positive linear effect of soil water content, at constant soil temperature, on different genes abundance for each treatment are presented on Fig. 1. The linear model effects are presented as model predictions of average community's abundance together with 95% confidence interval for the average predictions. The interactions between treatment and water content as well as treatment and temperature were not statistically significant (Table 1) as illustrated by the parallel slopes, except for AOA (Fig. 1).

The reduction of the total extracted DNA due to the decreased soil water content, at constant T, was on average 1.8% with 95% confidence interval (0.9%, 2.8%) if the WC decreases for 1 mass % (Table 2, Fig. 1). As a consequence, the abundance of all studied communities also significantly decreased on average from 1.8% to 2.6% with a 1% decrease of WC under all treatments, with the exception of AOA showing the highest decrease of 3.8%, however only under MT 0–10.

The reduction of the total extracted DNA due to the increased soil temperature, at constant water content, was on average 10.2% (95% CI 5.6%, 14.8%) if the temperature increases by 1 °C. Again, the results for all studied microbial communities were very similar (decrease of 8.7%–11.6% per °C) under all treatments, with the exception of AOA (Table 2). In the case of AOA, the effect of temperature, at constant water content, was statistically significant only under CT 10–20 treatment with 21.6% reduction (Tables 1 and 2). It should be noted that measurement of soil temperature only at one location point at 5 cm soil depth has some limitations. Nevertheless, we decided to include average daily soil temperature in our mixed model, since the variable reflects global weather conditions during the vegetation period (average daily T_{soil} ranged from 17 to 30 °C). Moreover, we hypothesised that soil temperatures under MT and CT were similar during the examined period since the crop residues on the soil surface were almost equal, and thus the most influential factors were absorption of solar radiation and evaporation (Rasmussen, 1999; Muñoz-Romero et al., 2015).

In contrast to our second hypothesis that the effects of drought might be less pronounced under MT than CT, no significant differences were found in slopes of communities response to reduced soil water content (at constant temperature) and to increased soil temperature (at constant water content) between MT and CT. The exception was AOA response to water content under MT 0–10 and to T under CT 10–20 (Fig. 1e, Table 1). Despite that the interactions between treatment & water content, and between treatment & temperature were not

statistically significant (Table 1), presumably due to relative small number of measurements, the greatest slope was found under MT 0–10 for all the communities, which could suggest reduced microbial resistance to drought in comparison with other treatments. This could be explained by the fact that the topsoil of MT had a significantly higher water holding capacity than that of CT (24.8% and 22.2%, respectively; Kaurin et al., 2015) and could therefore host different microbial communities that are less adapted to drought. Similar observations have been reported for soils amended with organic residues and exposed to drought (Orwin and Wardle, 2005; Bastida et al., 2017).

Previous studies have shown that drought decreases bacterial growth/activity while fungal communities are more resistant likely due to the hyphal network that can cross air-filled soil pores to access water and nutrients (Bapiri et al., 2010; Yuste et al., 2011; Sheik et al., 2011; De Vries et al., 2012; Barnard et al., 2013; Canarini et al., 2017). In contrast, our results showed a significant reduction of fungal abundance in response to decreased water content. Kaisermann et al. (2015) even reported higher sensitivity of fungi than bacteria in non-extreme soil moisture variations. There are also contradictory reports for archaea; on the one hand, they are found to be stable under changing environmental conditions (Valentine, 2007; Ollivier et al., 2011) and, on the other hand, there are also studies reporting a detrimental effect of drought on their abundance (Tian et al., 2012; Kwon et al., 2013). These contradictory findings may be due to (i) the specificity of studied ecosystems, since natural communities are composed of organisms with very different life history traits, physiology and dispersal ability (Schimel et al., 2007; Manzoni et al., 2014; Banerjee et al., 2016), so it is unlikely that they will all respond to drought in a similar way; and (ii) differences in the experimental approaches among studies (Canarini et al., 2017). Besides, laboratory incubation studies usually include a pre-treatment of soil by sieving, a disruptive procedure affecting soil structure and aggregation, which could therefore change the interactive effect of drought stress and soil physical properties. A recent meta-analysis by Manzoni et al. (2012, 2014) suggested that, although different microbial communities may employ different strategies to cope with drought stress, as the soil dries to a certain water content threshold (*near-zero point*), the solute diffusion limitation (and not biological constraints) becomes the main driver of microbial activity. Based on datasets from laboratory microbial incubation studies, this threshold was estimated to be at very low water potential (below −14000 kPa; Manzoni et al., 2012). During the drought conditions observed from July 25th to August 8th, soil water potential was below the wilting point (−1500 kPa), however soil was not completely dry with average soil water contents of 13.2 and 19.7% of WHC under MT 0–10 and CT 0–10, respectively (which equals 3.3 and 4.4% of soil dry weight, respectively). Results of our field study are therefore not comparable with the results from the laboratory microbial incubation studies with in general more extreme drought conditions leading to the non-linear relationship between soil moisture and microbial groups, respiration and growth (Howard and Howard, 1993; Lennon et al., 2012; Manzoni et al., 2012; Meisner et al., 2017).

There are also large discrepancies among studies describing the influence of drought on ammonia-oxidizers likely due to the same limitations underlined above. Some studies have therefore demonstrated a higher sensitivity of AOA (Thion and Prosser, 2014), others a higher sensitivity of AOB (Adair and Schwartz, 2008; Gleeson et al., 2010), or even a similar sensitivity of AOA and AOB (Placella and Firestone, 2013). While it is recognized that AOA and AOB have different ecological niches, the identification of their respective drivers is difficult since no single factor can discriminate between these two groups (Prosser and Nicol, 2012; Schleper, 2010; Radl et al., 2015; Sterngren et al., 2015). Nevertheless, our results demonstrated that drought significantly decreased the abundance of both AOA and AOB, under both tillage regimes (Table 1, Fig. 1d–e, Fig. 2d–e). Moreover, the results also indicated that AOA were more responsive to changing soil water conditions as AOB under MT 0–10 (only), with the highest decrease in abundance (3.8% with a 1% decrease of water content; Table 2d-e), and to reduced soil temperature (only) under CT 10–20 (21.6% decrease with 1 °C increase). Interestingly, AOA were in contrast to other communities, not responsive to changes in soil temperature under MT at both soil depths. Decreases in AOB and AOA abundances were up to 19 fold and up to 40 fold, respectively compared to their initial abundances (Fig. 2d–e). Reduced soil water content, at constant temperature, reduced the abundance of AOA more under MT than under CT at both soil depths, and AOB more under MT 0–10 than under other treatments (Fig. 2d–e). Increased soil NH_4^+ concentrations in the topsoil of MT and CT at the driest sampling date (four weeks without rain), may be associated with reduced activity of nitrifiers as showed by Stark and Firestone (1995). However, in ecosystems well adapted to drought, nitrification could also continue at a high level during the dry season (Sullivan et al., 2012).

To the best of our knowledge, there have only been a few studies evaluating the effects of drought on the abundance of denitrifiers (Hartmann et al., 2013; Gschwendtner et al., 2014; Keil et al., 2015), which similarly to nitrifiers, suggest that the response of denitrifiers can vary according to the severity of the drought event. We found a significant decrease in the abundance of denitrifying communities under both tillage treatments in response to drought (Tables 1 and 2, Fig. 1f–i). The range of gene reductions were 4.6–13.3 fold for *nirK*, 5.4–17.2 fold *nirS*, 5.6–12.3 fold for *nos*ZI and 8.8–24.9 fold the initial size for *nos*ZII (Fig. 2f–i). These decreases being in the same range for both microbial guilds capable of producing and/or reducing N_2O, it is likely that only N-emissions would have been modified at the end of the drought but not the proportion of N_2O to N_2. However, in contrast to our first hypothesis, denitrifiers were not more resistant to drought than ammonia-oxidising microorganisms, which supports recent findings suggesting that the extent of functional redundancy has been overstated (Philippot et al., 2013)

3.3. Resilience of microbial communities after drought

Although drought significantly decreased the abundances of the studied communities, we found that they all reached their initial abundance 3 weeks after the end of the drought (Fig. 2). Communities in the studied area have already been subjected to intense spring and summer droughts in the past (ARSO, 2017) and may therefore have adapted to changing water regime and soil temperature (Fierer et al., 2003; Evans and Wallenstein, 2012; Kaisermann et al., 2017). However, what the mechanisms underlying the microbial drought-response (acclimation and/or inherent resistance) were and whether they differed between the two tillage systems, remains a question of interest.

Soil management enhancing carbon stocks has been proposed to improve functional resilience and therefore the stability of microbial communities under drought conditions (Griffiths and Philippot, 2013; Kaisermann et al., 2013; Phillips et al., 2015; Sun et al., 2017). The hypothesised mechanisms in general involve improved soil aggregation and increased substrate availability for microbial colonization and metabolic adjustment under unfavourable conditions. Our results showed that microbial communities (bacterial and archaeal N-cycling guilds as well as total prokaryotes and fungi) completely recovered after the end of an extreme drought event under both MT and CT treatments. It remains to be elucidated whether this resilience in response to soil rewetting actually mirroring *in situ* nutrient flows. Altogether, our results suggest that MT increased the abundances of all quantified genes in comparison with CT but the tillage regime had no influence on the resilience of the studied communities to drought.

4. Conclusions

Nitrogen cycling is a key process in a soil ecosystem, although our understanding of how microbial communities driving this cycle respond to disturbances, such as drought, is still limited. Our study was the first

to the best of our knowledge to quantify differences in the response of nitrifier and denitrifier communities under different tillage regimes to a severe agricultural drought event. We found that the abundance of most studied microbial communities decreased by about 2% when WC decreases by 1 mass % and by about 10% when T increases by 1 °C. After three weeks of drought, the greatest decrease in the abundance of all communities was observed in the top soil under minimum tillage. However, under both tillage systems, all studied microbial communities reached their initial abundance 3 weeks after the end of drought, which suggests a high resilience. Greater efforts are needed for understanding how agricultural practices can modulate the response of key functional microbial communities to climate change.

Acknowledgements

This study was financially supported by the European Commission within the EcoFINDERS project (FP7-264465), FP7 ERA-net and CORE Organic Plus funding bodies within the FertilCrop project, and by the Slovenian Research Agency within the programme P4-0085.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2018.02.007.

References

Adair, K.L., Schwartz, E., 2008. Evidence that ammonia-oxidizing archaea are more abundant than ammonia-oxidizing bacteria in semiarid soils of northern Arizona, USA. Microbial Ecology 56, 420–426. http://dx.doi.org/10.1007/s00248-007-9360-9.

ARSO, 2017. Slovenian Agrometeorological Information Service. http://meteo.arso.gov.si/met/sl/agromet/.

Attard, E., Recous, S., Chabbi, A., De Berranger, C., Guillaumaud, N., Labreuche, J., Philippot, L., Schmid, B., Le Roux, X., 2011. Soil environmental conditions rather than denitrifier abundance and diversity drive potential denitrification after changes in land uses. Global Change Biology 17, 1975–1989. http://dx.doi.org/10.1111/j.1365-2486.2010.02340.x.

Banerjee, S., Helgason, B., Wang, L., Winsley, T., Ferrari, B.C., Siciliano, S.D., 2016. Legacy effects of soil moisture on microbial community structure and N2O emissions. Soil Biology and Biochemistry 95, 40–50. https://doi.org/10.1016/j.soilbio.2015.12.004.

Bapiri, A., Bååth, E., Rousk, J., 2010. Drying-rewetting cycles affect fungal and bacterial growth differently in an arable soil. Microbial Ecology 60, 419–428. http://dx.doi.org/10.1007/s00248-010-9723-5.

Barnard, R.L., Osborne, C.A., Firestone, M.K., 2013. Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. The ISME Journal 7, 2229–2241. http://dx.doi.org/10.1038/ismej.2013.104.

Bastida, F., Torres, I.F., Hernandez, T., García, C., 2017. The impacts of organic amendments: do they confer stability against drought on the soil microbial community? Soil Biology and Biochemistry 113, 173–183. https://doi.org/10.1016/j.soilbio.2017.06.012.

Baudoin, E., Philippot, L., Chèneby, D., Chapuis-Lardy, L., Fromin, N., Bru, D., Rabary, B., Brauman, A., 2009. Direct seeding mulch-based cropping increases both the activity and the abundance of denitrifier communities in a tropical soil. Soil Biology and Biochemistry 41, 1703–1709. http://dx.doi.org/10.1016/j.soilbio.2009.05.015.

Bru, D., Ramette, A., Saby, N.P., Dequiedt, S., Ranjard, L., Jolivet, C., Arrouays, D., Philippot, L., 2011. Determinants of the distribution of nitrogen-cycling microbial communities at the landscape scale. The ISME Journal 5, 532–542. http://dx.doi.org/10.1038/ismej.2010.130.

Busari, M.A., Kukal, S.S., Kaur, A., Bhatt, R., Dulazi, A.A., 2015. Conservation tillage impacts on soil, crop and the environment. International Soil and Water Conservation Research 3, 119–129. https://doi.org/10.1016/j.iswcr.2015.05.002.

Cammalleri, C., Micale, F., Vogt, J., 2016. A novel soil moisture-based drought severity index (DSI) combining water deficit magnitude and frequency. Hydrological Processes 30, 289–301.

Canarini, A., Kiær, L.P., Dijkstra, F.A., 2017. Soil carbon loss regulated by drought intensity and available substrate: a meta-analysis. Soil Biology and Biochemistry 112, 90–99. https://doi.org/10.1016/j.soilbio.2017.04.020.

Cruz-Martínez, K., Suttle, K.B., Brodie, E.L., Power, M.E., Andersen, G.L., Banfield, J.F., 2009. Despite strong seasonal responses, soil microbial consortia are more resilient to long-term changes in rainfall than overlying grassland. The ISME Journal 3, 738–744. http://dx.doi.org/10.1038/ismej.2009.16.

Dai, A., 2012. Increasing drought under global warming in observations and models. Nature Climate Change 3, 52–58. http://dx.doi.org/10.1038/NCLIMATE1633.

De Vries, F.T., Liiri, M.E., Bjornlund, L., Bowker, M.A., Christensen, S., Setala, H.M., Bardgett, R.D., 2012. Land use alters the resistance and resilience of soil food webs to drought. Nature Climate Change 2, 276–280. http://dx.doi.org/10.1038/NCLIMATE1368.

Domeignoz-Horta, L.A., Spor, A., Bru, D., Breuil, M.C., Bizouard, F., Léonard, J., Philippot, L., 2015. The diversity of the N2O reducers matters for the N2O: N2 denitrification end-product ratio across an annual and a perennial cropping system. Frontiers in Microbiology 6, 971. http://dx.doi.org/10.3389/fmicb.2015.00971.

Domeignoz-Horta, L.A., Putz, M., Spor, A., Bru, D., Breuil, M.C., Hallin, S., Philippot, L., 2016. Non-denitrifying nitrous oxide-reducing bacteria - an effective N2O sink in soil. Soil Biology and Biochemistry 103, 376–379. http://dx.doi.org/10.1016/j.soilbio.2016.09.010.

Evans, S.E., Wallenstein, M.D., 2012. Soil microbial community response to drying and rewetting stress: does historical precipitation regime matter? Biogeochemistry 109, 101–116. http://dx.doi.org/10.1007/s10533-011-9638-3.

Fierer, N., Schimel, J.P., Holden, P.A., 2003. Influence of drying-rewetting frequency on soil bacterial community structure. Microbial Ecology 45, 63–71. http://dx.doi.org/10.1007/s00248-002-1007-2.

Fuchslueger, L., Kastl, E.M., Bauer, F., Kienzl, S., Hasibeder, R., Ladreiter-Knauss, T., Schmitt, M., Bahn, M., Schloter, M., Richter, A., Szukics, U., 2014. Effects of drought on nitrogen turnover and abundances of ammonia-oxidizers in mountain grassland. Biogeosciences 11, 6003–6015. http://dx.doi.org/10.5194/bg-11-6003-2014.

Gleeson, D.B., Müller, C., Banerjee, S., Ma, W., Siciliano, S.D., Murphy, D.V., 2010. Response of ammonia oxidizing archaea and bacteria to changing water filled pore space. Soil Biology and Biochemistry 42, 1888–1891. http://dx.doi.org/10.1016/j.soilbio.2010.06.020.

Griffiths, R.I., Whiteley, A.S., Anthony, G., Donnell, O., Bailey, M.J., Donnell, A.G.O., 2003. Physiological and community responses of established grassland bacterial populations to water stress physiological and community responses of established grassland bacterial populations to water stress. Applied and Environmental Microbiology 69, 6961–6968. http://dx.doi.org/10.1128/AEM.69.12.6961.

Griffiths, R.I., Philippot, L., 2013. Insights into the resistance and resilience of the soil microbial community. FEMS Microbiology Reviews 37, 112–129. http://dx.doi.org/10.1111/j.1574-6976.2012.00343.x.

Gubry-Rangin, C., Nicol, G.W., Prosser, J.I., 2010. Archaea rather than bacteria control nitrification in two agricultural acidic soils. FEMS Microbiology Ecology 74, 566–574. http://dx.doi.org/10.1111/j.1574-6941.2010.00971.x.

Gschwendtner, S., Tejedor, J., Bimueller, C., Dannenmann, M., Kögel Knabner, I., Schloter, M., 2014. Climate change induces shifts in abundance and activity pattern of bacteria and archaea catalyzing major transformation steps in nitrogen turnover in a soil from a Mid-European beech forest. PLoS One 9 e116614. https://doi.org/10.1371/journal.pone.0116614.

Hartmann, A.A., Barnard, R.L., Marhan, S., Niklaus, P.A., 2013. Effects of drought and N-fertilization on N cycling in two grassland soils. Oecologia 171, 705–717. http://dx.doi.org/10.1007/s00442-012-2578-3.

He, J.-Z., Shen, J.-P., Zhang, L.-M., Zhu, Y.-G., Zheng, Y.-M., Xu, M.-G., Di, H., 2007. Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices. Environmental Microbiology 9, 2364–2374. http://dx.doi.org/10.1111/j.1462-2920.2007.01358.x.

Helgason, B.L., Walley, F.L., Germida, J.J., 2009. Fungal and bacterial abundance in long-term no-till and intensive-till soils of the Northern Great Plains. Soil Science Society of America Journal 73, 120–127. http://dx.doi.org/10.2136/sssaj2007.0392.

Helgason, B.L., Walley, F.L., Germida, J.J., 2010. No-till soil management increases microbial biomass and alters community profiles in soil aggregates. Applied Soil Ecology 46, 390–397. http://dx.doi.org/10.1016/j.apsoil.2010.10.002.

Henry, S., Baudoin, E., López-Gutiérrez, J.C., Martin-Laurent, F., Brauman, A., Philippot, L., 2004. Quantification of denitrifying bacteria in soils by nirK gene targeted real-time PCR. Journal of Microbiological Methods 59, 327–335. http://dx.doi.org/10.1016/j.mimet.2004.07.002.

Henry, S., Bru, D., Stres, B., Hallet, S., Philippot, L., 2006. Quantitative detection of the nosZ gene, encoding nitrous oxide reductase, and comparison of the abundances of 16S rRNA, narG, nirK, and nosZ genes in soils. Applied and Environmental Microbiology 72, 5181–5189. http://dx.doi.org/10.1128/AEM.00231-06.

Howard, D.M., Howard, P.J.A., 1993. Relationships between CO2 evolution, moisture content and temperature for a range of soil types. Soil Biology and Biochemistry 25, 1537–1546.

Hydbom, S., Ernforsc, M., Birganderb, J., Hollanderb, J., Jensenc, E.S., Olsson, P.A., 2017. Reduced tillage stimulated symbiotic fungi and microbial saprotrophs, but did not lead to a shift in the saprotrophic microorganism community structure. Applied Soil Ecology 119, 104–114. https://doi.org/10.1016/j.apsoil.2017.05.032.

IPCC, 2013. Climate Change 2013: the Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Intergovernmental Panel on Climate Change, Working Group I Contribution to the IPCC Fifth Assessment Report (AR5). Cambridge Univ Press, New York, pp. 1535. http://dx.doi.org/10.1029/2000JD000115.

ISO 11274, 1998. Soil Quality - Determination of the Water Retention Characteristic, Laboratory Methods. International Organization for Standardization, Genève, Switzerland.

ISO 11465, 1993. Soil Quality - Determination of Dry Matter and Water Content on a Mass Basis - Gravimetric Method. International Organization for Standardization, Genève, Switzerland.

Jia, Z., Conrad, R., 2009. Bacteria rather than Archaea dominate microbial ammonia oxidation in an agricultural soil. Environmental Microbiology 11, 1658–1671. http://dx.doi.org/10.1111/j.1462-2920.2009.01891.x.

Jones, C.M., Graf, D.R.H., Bru, D., Philippot, L., Hallin, S., 2013. The unaccounted yet abundant nitrous oxide-reducing microbial community: a potential nitrous oxide sink. The ISME Journal 7, 417–426. http://dx.doi.org/10.1038/ismej.2012.125.

Kaisermann, A., Roguet, A., Nunan, N., Maron, P.A., Ostle, N., Lata, J.C., 2013. Agricultural management affects the response of soil bacterial community structure and respiration to water-stress. Soil Biology and Biochemistry 66, 69–77. http://dx.doi.org/10.1016/j.soilbio.2013.07.001.

Kaisermann, A., Maron, P.A., Beaumelle, L., Lata, J.C., 2015. Fungal communities are more sensitive indicators to non-extreme soil moisture variations than bacterial communities. Applied Soil Ecology 86, 158–164. https://doi.org/10.1016/j.apsoil.2014.10.009.

Kaisermann, A., de Vries, F.T., Griffiths, R.I., Bardgett, R.D., 2017. Legacy effects of drought on plant–soil feedbacks and plant–plant interactions. New Phytologist 215, 1413–1424. http://dx.doi.org/10.1111/nph.14661.

Kandeler, E., Deiglmayr, K., Tscherko, D., Bru, D., Philippot, L., 2006. Abundance of narG, nirS, nirK, and nosZ genes of denitrifying bacteria during primary successions of a glacier foreland. Applied and Environmental Microbiology 72, 5957–5962. http://dx.doi.org/10.1128/AEM.00439-06.

Kaurin, A., Mihelič, R., Kastelec, D., Schloter, M., Suhadolc, M., Grčman, H., 2015. Consequences of minimum soil tillage on abiotic soil properties and composition of microbial communities in a shallow Cambisol originated from fluvioglacial deposits. Biology and Fertility of Soils 51, 923–933. http://dx.doi.org/10.1007/s00374-015-1037-9.

Keil, D., Niklaus, P.A., von Riedmatten, L.R., Boeddinghaus, R.S., Dormann, C.F., Scherer-Lorenzen, M., Kandeler, E., Marhan, S., 2015. Effects of warming and drought on potential N2O emissions and denitrifying bacteria abundance in grasslands with different land-use. FEMS Microbiology Ecology 91, 1–9. http://dx.doi.org/10.1093/femsec/fiv066.

Krauss, M., Krause, H.-M., Spangler, S., Kandeler, E., Behrens, S., Kappler, A., Mäder, P., Gattinger, A., 2016. Tillage system affects fertilizer-induced nitrous oxide emissions. Biology and Fertility of Soils 53, 49–59. http://dx.doi.org/10.1007/s00374-016-1152-2.

Kwon, M.J., Haraguchi, A., Kang, H., 2013. Long-term water regime differentiates changes in decomposition and microbial properties in tropical peat soils exposed to the short-term drought. Soil Biology and Biochemistry 60, 33–44. http://dx.doi.org/10.1016/j.soilbio.2013.01.023.

Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G.W., Prosser, J.I., Schuster, S.C., Schleper, C., 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442, 806–809. http://dx.doi.org/10.1038/nature04983.

Lennon, J.T., Aanderud, Z.T., Lehmkuhl, B.K., Schoolmaster, D.R., 2012. Mapping the niche space of soil microorganisms using taxonomy and traits. Ecology 93, 1867–1879.

Manzoni, S., Schimel, J.P., Porporato, A., 2012. Responses of soil microbial communities to water stress: results from a meta-analysis. Ecology 93, 930–938.

Manzoni, S., Schaeffer, S.M., Katul, G., Porporato, A., Schimel, J.P., 2014. A theoretical analysis of microbial eco-physiological and diffusion limitations to carbon cycling in drying soils. Soil Biology and Biochemistry 73, 69–83. https://doi.org/10.1016/j.soilbio.2014.02.008.

Meisner, A., Leizeaga, A., Rousk, J., Bååth, E., 2017. Partial drying accelerates bacterial growth recovery to rewetting. Soil Biology and Biochemistry 112, 269–276.

Muñoz-Romero, V., Lopez-Bellido, L., Lopez-Bellido, R.J., 2015. Effect of tillage system on soil temperature in a rainfed Mediterranean Vertisol. International Agrophysics 29, 467–473. http://dx.doi.org/10.1515/intag-2015-0052.

Muyzer, G., De Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied and Environmental Microbiology 59, 695–700.

Ochsenreiter, T., Selezi, D., Quaiser, A., Bonch-Osmolovskaya, L., Schleper, C., 2003. Diversity and abundance of Crenarchaeota in terrestrial habitats studied by 16S RNA surveys and real time PCR. Environmental Microbiology 5, 787–797. http://dx.doi.org/10.1046/j.1462-2920.2003.00476.x.

Ollivier, J., Owe, S., Bannert, A., Hai, B., Kastl, E.-M., Meyer, A., Su, M.X., Kleineidam, K., Schloter, M., 2011. Nitrogen turnover in soil and global change. FEMS Microbiology Ecology 78, 3–16. http://dx.doi.org/10.1111/j.1574-6941.2011.01165.x.

Orwin, K.H., Wardle, D.A., 2005. Plant species composition effects on belowground properties and the resistance and resilience of the soil microflora to a drying disturbance. Plant and Soil 278, 205–221. http://dx.doi.org/10.1007/s11104-005-8424-1.

Perez-Brandán, C., Arzeno, J.L., Huidobro, J., Grümberg, B., Conforto, C., Hilton, S., Bending, G.D., Meriles, J.M., Vargas-Gil, S., 2012. Long-term effect of tillage systems on soil microbiological, chemical and physical parameters and the incidence of charcoal rot by Macrophomina phaseolina (Tassi) Goid in soybean. Crop Protection 40, 73–82. http://dx.doi.org/10.1016/j.cropro.2012.04.018.

Philippot, L., Hallin, S., Schloter, M., 2007. Ecology of denitrifying prokaryotes in agricultural soil. Advances in Agronomy 96, 249–305. http://dx.doi.org/10.1016/S0065-2113(07)96003-4.

Philippot, L., Spor, A., Henault, C., Bru, D., Bizouard, F., Jones, C.M., Sarr, A., Maron, P.A., 2013. Loss in microbial diversity affects nitrogen cycling in soil. The ISME Journal 7, 1609–1619. http://dx.doi.org/10.1038/ismej.2013.34.

Phillips, L.A., Schefe, C.R., Fridman, M., O'Halloran, N., Armstrong, R.D., Mele, P.M., 2015. Organic nitrogen cycling microbial communities are abundant in a dry Australian agricultural soil. Soil Biology and Biochemistry 86, 201–211. http://dx.doi.org/10.1016/j.soilbio.2015.04.004.

Placella, S.A., Firestone, M.K., 2013. Communities to wetting of dry soil transcriptional response of nitrifying. Applied and Environmental Microbiology 10, 3294–3302. http://dx.doi.org/10.1128/AEM.00404-13.

Prosser, J.I., Nicol, G.W., 2008. Relative contributions of archaea and bacteria to aerobic ammonia oxidation in the environment. Environmental Microbiology 10, 2931–2941. http://dx.doi.org/10.1111/j.1462-2920.2008.01775.x.

Prosser, J.I., Nicol, G.W., 2012. Archaeal and bacterial ammoniaoxidisers in soil: the quest for niche specialisation and differentiation. Trends in Microbiology 11, 523–531. https://doi.org/10.1016/j.tim.2012.08.001.

R Core Team, 2017. A Language and Environment for Statistical Computing. R foundation for statistical computing, Vienna, Austria. http://www.R-project.org.

Radl, V., Kindler, R., Welzl, G., Albert, A., Wilke, B.M., Amelung, W., Schloter, M., 2015. Drying and rewetting events change the response pattern of nitrifiers but not of denitrifiers to the application of manure containing antibiotic in soil. Applied Soil Ecology 95, 99–106. https://doi.org/10.1016/j.apsoil.2015.06.016.

Rasmussen, K.J., 1999. Impact of ploughless soil tillage on yield and soil quality: a Scandinavian review. Soil and Tillage Research 53, 3–14.

Schimel, J.P., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 86, 1386–1394.

Schleper, C., 2010. Ammonia oxidation: different niches for bacteria and archaea? The ISME Journal 4, 1092–1094. http://dx.doi.org/10.1038/ismej.2010.111.

Sheik, C.S., Beasley, W.H., Elshahed, M.S., Zhou, X., Luo, Y., Krumholz, L.R., 2011. Effect of warming and drought on grassland microbial communities. The ISME Journal 5, 1692–1700. http://dx.doi.org/10.1038/ismej.2011.32.

Sims, A., Horton, J., Gajaraj, S., Mcintosh, S., Miles, R.J., Mueller, R., Reed, R., Hu, Z., 2012. Temporal and spatial distributions of ammonia-oxidizing archaea and bacteria and their ratio as an indicator of oligotrophic conditions in natural wetlands. Water Research 46, 4121–4129. http://dx.doi.org/10.1016/j.watres.2012.05.007.

Stark, J.M., Firestone, M.K., 1995. Mechanisms for soil moisture effects on activity of nitrifying bacteria. Applied and Environmental Microbiology 61, 218–221.

Strauss, S.L., Reardon, C.L., Mazzola, M., 2014. The response of ammonia-oxidizer activity and community structure to fertilizer amendment of orchard soils. Soil Biology and Biochemistry 68, 410–418. http://dx.doi.org/10.1016/j.soilbio.2013.10.016.

Sterngren, A.E., Hallin, S., Bengtson, P., 2015. Archaeal ammonia oxidizers dominate in numbers, but bacteria drive gross nitrification in N-amended grassland soil. Frontiers in Microbiology 2015 (6), 1350. http://dx.doi.org/10.3389/fmicb.2015.01350.

Sullivan, B.W., Selmants, P.C., Hart, S.C., 2012. New evidence that high potential nitrification rates occur in soils during dry seasons: are microbial communities metabolically active during dry seasons? Soil Biology and Biochemistry 53, 28–31. http://dx.doi.org/10.1016/j.soilbio.2012.04.029.

Sun, B., Jia, S., Zhang, S., McLaughlin, N.B., Zhang, X., Liang, A., Chen, X., Wei, S., Liu, S., 2016. Tillage, seasonal and depths effects on soil microbial properties in black soil of Northeast China. Soil and Tillage Research 155, 421–428. http://dx.doi.org/10.1016/j.still.2015.09.014.

Sun, D., Li, K., Bi, Q., Zhu, J., Zhang, Q., Jin, C., Lu, L., Lin, X., 2017. Effects of organic amendment on soil aggregation and microbial community composition during drying-rewetting alternation. The Science of the Total Environment 574, 735–743. https://doi.org/10.1016/j.scitotenv.2016.09.112.

Sušnik, A., 2014. Zasnove Kazalcev Spremljanja Suše Na Kmetijskih Površinah/Scheme of Indicators for Drought Monitoring on Agricultural Land. University of Ljubljana, Ljubljana. http://www.digitalna-knjiznica.bf.uni-lj.si/agronomija/dd_susnik_andreja.pdf.

Sušnik, A., Valher, A., 2014. Od mokre pomladi do sušnega poletja 2013. From wet spring to severe summer drought of 2013. UJMA 28, 75–84.

Tabaglio, V., Gavazzi, C., Menta, C., 2009. Physico-chemical indicators and microarthropod communities as influenced by no-till, conventional tillage and nitrogen fertilisation after four years of continuous maize. Soil and Tillage Research 105, 135–142. http://dx.doi.org/10.1016/j.still.2009.06.006.

Thion, C., Prosser, J.I., 2014. Differential response of non-adapted ammonia oxidising archaea and bacteria to drying rewetting stress. FEMS Microbiology Ecology 1–10. http://dx.doi.org/10.1111/1574-6941.12395.

Tian, J., Zhu, Y., Kang, X., Dong, X., Li, W., Chen, H., Wang, Y., 2012. Effects of drought on the archaeal community in soil of the Zoige wetlands of the Qinghai-Tibetan plateau. European Journal of Soil Biology 52, 84–90. http://dx.doi.org/10.1016/j.ejsobi.2012.07.003.

Tourna, M., Freitag, T.E., Nicol, G.W., Prosser, J.I., 2008. Growth, activity and temperature responses of ammonia-oxidizing archaea and bacteria in soil microcosms. Environmental Microbiology 10, 1357–1364. http://dx.doi.org/10.1111/j.1462-2920.2007.01563.x.

Valentine, D.L., 2007. Adaptations to energy stress dictate the ecology and evolution of the Archaea. Nature Reviews Microbiology 5, 316–323. http://dx.doi.org/10.1038/nrmicro1619.

Wardle, D.A., 1995. Impacts on detritus food webs in agro-ecosystems of contrasting tillage and weed management practices. Advances in Ecological Research 26, 105–182.

White, P.M., Rice, C.W., 2009. Tillage effects on microbial and carbon dynamics during plant residue decomposition. Soil Science Society of America Journal 73, 138. http://dx.doi.org/10.2136/sssaj2007.0384.

White, T.J., Bruns, S., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M., Gelfand, D., Sninsky, J., White, T. (Eds.), PCR Protocols: a Guide to Methods and Applications. Academic Press, Orlando, Florida, pp. 315–322 doi:citeulike-article-id:671166.

Yaduvanshi, N.P.S., Sharma, D.R., 2008. Tillage and residual organic manures/chemical amendment effects on soil organic matter and yield of wheat under sodic water irrigation. Soil and Tillage Research 1, 11–16. http://dx.doi.org/10.1016/j.still.2007.09.010.

Yuste, J.C., Penuelas, J., Estiarte, M., Garcia-Masw, J., Mattana, S., Ogaya, R., Pujol, M., Sardans, J., 2011. Drought-resistant fungi control soil organic matter decomposition and its response to temperature. Global Change Biology 17, 1475–1486. http://dx.doi.org/10.1111/j.1365-2486.2010.02300.x.